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Coronavirus Pandemic Jumpstarts Wastewater-Based Epidemiology

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NEW YORK – Like performing one giant pooling test, assessing sewage for SARS-CoV-2 has the potential to localize hotspots of COVID-19 infection. Municipalities around the world are now beginning to scale up nascent wastewater-based epidemiology programs using standard commercially available extraction and PCR kits, as well as new products designed for SARS-CoV-2 detection from wastewater, and start-ups have begun offering testing and epidemiology consulting services.

Monitoring for a wisp of RNA virus in liter-sized volumes of raw sewage is no small feat, and might just take the cake in terms of PCR inhibitors and nucleic acid degradation. The field of molecular wastewater-based epidemiology (WBE) is also relatively new, so in parallel to the push to rapidly deploy SARS-CoV-2 sewage surveillance, researchers around the world are hammering out the basic protocols to best sample wastewater, extract and detect viral nucleic acids, and correlate viral loads in wastewater with human infection trends.

There is increasing consensus around the idea that wastewater monitoring is a powerful tool to help direct the resources of cash-strapped public health departments. However, in the US the scale up of sewage monitoring seems to be currently left to local governments. The National Science Foundation and others have recently flushed the field with funding for basic research, but some experts suggest a coordinated federal effort is also needed to more effectively bring WBE to bear on the COVID-19 pandemic.

Although environmental surveillance of wastewater has been deployed to pinpoint and monitor local polio and hepatitis outbreaks, in general the field of WBE is thought to have kicked off with a 2001 study hypothesizing the method could be used to monitor illicit drug use in a population, according to Adam Gushgari, a civil engineer and co-founder of AquaVitas, a startup in the WBE space.

WBE was subsequently demonstrated to be feasible in 2005, when clinical chemistry techniques were used to detect a cocaine metabolite in wastewater from a few Italian cities. The technique has been further adapted in the US for opioid surveillance in cities and college campuses.

Although narcotics had been the main focus of the WBE, interest in molecular pathogen detection has been growing, particularly over the past three years, Gushgari said. And now, "The field has seen a definite increase in interest because of the pandemic," he said in an interview.

But, wastewater remains "an extremely complex — if not the most complex — environmental matrix that we can use as a diagnostic tool," he said, so it takes a lot of trial and error to balance the extreme extraction required with preservation of target integrity.

In the developing research space and applied market of WBE, there are many interconnected networks of players.

AquaVitas spun out of the Biodesign Center for Environmental Health Engineering at Arizona State University and was co-founded by WBE expert and center Director Rolf Halden.

Halden and his team at ASU also support one of the most mature wastewater-related public health efforts in the US in the city of Tempe, Arizona. There, they have run testing and coordinated a publicly-facing online opiate monitoring dashboard since 2018.

Now, the ASU team has scaled this project to create a SARS-CoV-2 dashboard to surveil and report on seven areas of Tempe, monitoring overall infection trends among approximately 185,000 local residents.

Both the opiate and COVID dashboards in Tempe are integrated into public health management, Halden said in an email, and his team meets monthly with the mayor's office to help identify areas of concern and direct limited resources where they are needed most.

In a recent study, Halden and his collaborator Olga Hart illuminated both the challenges and the potential economic opportunities of WBE through modeling of eight global SARS-CoV-2 hotspots. The study estimated that incorporating national sewage surveillance campaigns could potentially save billions of dollars compared to clinical diagnostic testing alone.

Likewise, the author of the seminal 2001 WBE study, Christian Daughton, who recently retired from the Environmental Protection Agency, penned a review in May arguing national approaches to SARS-CoV-2 sewage surveillance could be beneficial in part because large-scale diagnostic testing of individuals faces "overwhelming challenges in providing fast surveys of large populations." In contrast, WBE could help not only in mitigation and containment of the pandemic, but also could — hopefully — minimize "domino effects, such as unnecessarily long stay-at-home policies that stress humans and economies alike," Daughton wrote.

A handful of US municipalities seem to have been persuaded already. Besides Tempe, pilot programs in Utah and Montana are currently generating coronavirus wastewater monitoring data and are sharing it with the public. And, a WBE project supported by the EPA is slated to be rolled out across the state of Ohio in the near future.

Perhaps emblematic of the parallel tracks that seem to be part of this blossoming space, EPA's researchers are continuing efforts to develop analytical methods, even as the program is being rolled out.

Melissa Sullivan in EPA's office of public affairs commented in an email that the agency's researchers are currently working on methods to measure SARS-CoV-2 virus in wastewater, as well as establishing techniques for "interpreting the data generated by these evolving analytical methods and developing models to predict community COVID infection rates."

In a related effort, Sullivan said EPA is contributing data and working to finalize a plan for a statewide wastewater monitoring program in Ohio. That program includes collaborators from the Metropolitan Sewer District of Greater Cincinnati, the Ohio Department of Health, the Ohio EPA, and the Ohio Water Resources Center at the Ohio State University.

Internationally, pre-existing wastewater surveillance programs may be more deeply rooted than in the US, so SARS-CoV-2 WBE seems to be taking off at somewhat of a faster pace, albeit with identical technical hurdles.

At the National Academies' Water Science and Technology Board virtual annual meeting in May, Gertjan Medema, a researcher at Delft University of Technology, pointed out that the Netherlands, Finland, and Germany are examples of countries that have already begun the groundwork to implement national SARS-CoV-2 surveillance programs, with dozens of sentinel cities currently being surveilled.

Meanwhile, a longstanding European wastewater monitoring program called the Sewage Analysis Core group Europe, or SCORE, is adapting from a focus on drugs-of-abuse surveillance to SARS-CoV-2 WBE.

Launched in 2011, SCORE currently tracks sewage from 86 cities in 29 countries. Lian Lundy, an environmental scientist at Middlesex University London, is helping to lead two related SCORE initiatives in the EU for SARS-CoV-2. One is an overall feasibility assessment in which all samples will be analyzed in a single lab, Lundy said in an email, while the second supports the development of an international open-access database.

"So much is happening so fast," Lundy said, and the key challenge currently is that there is "no standard analytical method" for SARS-CoV-2 detection in wastewater. She noted that on average it takes approximately three years for standardization organizations within the water sciences field to approve a method. Lundy suggested, however, that it might be possible to address this challenge through use of a robust QA/QC framework.

Methods and vendors

Research teams around the world are indeed sharing their WBE protocols and sewage solutions, and some are collaborating extensively to essentially crowdsource elaborate protocol troubleshooting.

Overall, in the burgeoning body of literature on SARS-CoV-2 WBE, the methods used for PCR-based testing are essentially the standard methods used by clinical and research labs, with some of the front-end sample prep perhaps needing extra finesse.

One particular method for SARS-CoV-2 molecular detection in wastewater that seems to be a starting place for many local teams stems from pioneering work out of Australia by Warish Ahmed and his colleagues at the Commonwealth Scientific and Industrial Research Organisation.

Ahmed uses electronegative membranes to capture viruses and then extracts RNA directly from the membranes using Qiagen PowerWater or PowerMicrobiome extraction kits, he said in an email. He then uses either a Bio-Rad CFX-96 thermal cycler or digital PCR to quantify SARS-CoV-2 RNA in wastewater. For the latter, he noted that he recently ordered a Qiagen dPCR platform because he found it to be especially fast.

Ahmed also recently co-authored a paper in *Environmental Science and Technology* laying the groundwork for a global collaborative effort with SCORE and the Global Water Pathogen Project that hopes to empower worldwide wastewater-based epidemiology of SARS-CoV-2.

In Australia, Ahmed said he and his team are in touch with public health officials and inform them of the WBE results to help them make decisions.

In Tempe, meanwhile, Halden said his team at ASU uses Ahmed's basic method for the detection of SARS-CoV-2 in municipal wastewater, specifically using standard qPCR probes that have been published and validated by the US Centers for Disease Control and the World Health Organization. The ASU team has also demonstrated temperature sensitivity and in-sewer decay of SARS-CoV-2 targets, Halden said, and so they are using "proprietary methods and devices" to prevent and compensate for these challenges. Halden and his team have opted not to use digital PCR at this time, based on prior experiences with the technology, he said.

In terms of technical challenges, Ahmed noted that if the concentration of virus in wastewater is high, detection is pretty straightforward. But, "when the concentration of virus is low, it can be challenging to detect." To combat this issue, labs can use a better recovery method or perform more replicates in the PCR reactions, he said. Ahmed's team is now working on several potential refinements, such as comparing different platforms, and single time-point analysis — also called "grab sampling" — to analyze samples of wastewater pooled over extended periods of time.

According to Halden, other technical aspects will require still more protocol refinements.

"Wastewater monitoring sounds simple, but in practice there are many challenges that require careful planning," he said, including sampling, shipping, analysis, and sample archiving. The ASU team has developed techniques and technologies to overcome signal decay, for example, and has quantified the ways temperature effects virual decay and the impact of the effective size of the monitoring network. Another issue is that automated sewage samplers that are supposed to deliver 24-hour composite samples in actuality may sample only a few minutes of each hour, Halden said, resulting in signal passing through the monitoring location uncaptured and unaccounted for. "We have developed customized equipment that improves signal capture and sample representativeness by over an order of magnitude," Halden said.

Once the best possible sample is captured, most protocols for the PCR lab work appear to use methods to capture and concentrate the nucleic acids in raw wastewater, often followed by off-the-shelf extraction and reagent kits — sometimes specifically designed for wastewater samples — and standard PCR instruments.

For example, some researchers are using the aforementioned Qiagen extraction kits designed for environmental testing of soil or water, although the firm also markets the AllPrep PowerViral DNA/RNA Kit for samples high in PCR inhibitors like wastewater. And, in a preliminary study, Medema and colleagues at Delft University centrifuged and filterer sewage then used magnetic extraction with the BioMérieux Nuclisens kit in combination with Thermo Fisher's semi-automated high-thoughput KingFisher system.

Meanwhile, other reagent manufacturers are now jumping in with products purposely designed for SARS-CoV-2 detection from wastewater.

Madison, Wisconsin-based Promega had already been offering a system to purify viral RNA from concentrated wastewater using the Maxwell RSC, a magnetic particle handler capable of processing 1-16 or 1-48 samples simultaneously and its PureFood GMO and Authentication Kit for nucleic acid extraction from wastewater samples, according to Brigitta Saul, Promega's global commercialization marketing manager.

Promega has also now developed protocols specifically for detecting SARS-CoV-2 RNA in wastewater samples, namely for viral purification using either a type of precipitation or centrifugal filtration.

Saul said Promega has a viral RNA purification chemistry under development, too. The kit is for wastewater samples and can be performed for single samples using manual extraction, but is also adaptable to higher-throughput automated RT-qPCR detection. The product includes primers and probes for SARS-CoV-2 detection, as well as suitable amplification controls. "This kit is currently undergoing early testing by potential users," Saul said.

Promega's "expertise on the design of nucleic acid purification systems and formulating PCR master mixes tolerant of inhibitors commonly found in environmental samples" allows the firm "to design and formulate workflow solutions that are able to cope with these kinds of samples," Saul noted.

Thermo Fisher Scientific is also extrapolating an existing footprint in WBE to more purpose-designed products. Specifically, a representative said the firm's commercially available next-generation sequencing product is being used for epidemiological studies of SARS-CoV-2, and the Ion AmpliSeq SARS-CoV-2 Research Panel is also being used by some of Thermo Fisher's customers to analyze the virus in waste water.

Studies, startups, and scale-up

An early adopter of Thermo Fisher's technology also happens to be a good illustration of WBE's hyper-local roots.

Paraic Kenny is the director of the Kabara Cancer Research Institute at Gundersen Health System in La Crosse, Wisconsin, a city with a population of approximately 50,000 people.

Kenny and his colleagues have been serially sampling local wastewater since early May, obtaining 24-hour composite collection samples, which they then concentrate and extract RNA from. They then use the Ion Ampliseq SARS-CoV-2 research panel from Thermo Fisher to amplify viral genomes and sequence libraries on the Ion Torrent S5, Kenny said in an email.

The goal is to monitor substrain prevalence in the community to help inform healthcare utilization prediction models, he said, so that the hospital system may be forewarned about imminent patient surges.

"Looking retrospectively, we could see a clear signal in early June for one particular variant that foreshadowed a substantial outbreak in La Crosse county that became evident in mid-June," Kenny noted, adding that "confidently predicting which newly observed variant might indicate an upcoming outbreak will require a lot more validation."

Kenny's efforts in La Crosse are supported by the Gundersen Medical Foundation, but he said his team is seeking additional funds elsewhere to ensure sustainability.

Fortunately, funding for SARS-CoV-2 WBE has grown exponentially in the past six months.

The National Science Foundation, for example, recently began distributing rapid funding for about a dozen basic research projects in the WBE field.

Karl Rockne, the program director for the NSF arm overseeing wastewater epidemiology projects, said in an email that the agency issued a COVID-related, NSF-wide "Dear Colleague Letter" in April, encouraging applications for a unique funding mechanisms called RAPID.

Rockne, who is the director of NSF's Environmental Engineering Program in the Division of Chemical, Bioengineering, Environmental, and Transport Systems, said the NSF received over 6,000 enquiries in response to the DCL, and the Environmental Engineering program funded approximately 20 awards covering WBE, building shutdowns, air sampling for SARS-CoV-2, and related research.

Put together, all of the COVID RAPID awards from the Environmental Engineering program exceed $3 million, Rockne said, although he did not offer a more detailed breakdown of the subtopics.

Rockne said that the funding came from NSF's normal core program funds and was supplemented from the pool of $75 million in CARES Act funds dedicated to NSF specifically for RAPID SARS-CoV-2 research projects. Projects in Oregon, Hawaii, and Ohio, for example, received initial awards of $100,000, $151,956, and $197,375, respectively, to work out basic WBE methodologies and protocols.

And while the NSF is funding basic research studies in "numerous communities across the country with different characteristics," Rockne said, in parallel to these local projects it is also funding a "Research Coordination Network" that aims to "put all the research teams together, so they can learn from each other to maximize the public benefit."

With new public and private funding sources, many research teams across the US have now launched projects to validate wastewater testing in different systems, using different methods.

For example, a team of researchers from the University Wisconsin-Milwaukee, New York University, Notre Dame, and Stanford University is using a recently awarded $250,000 grant from the Alfred P. Sloan Foundation to perfect methods to enable, in part, the implementation of a SARS-CoV-2 wastewater monitoring program in New York City.

Sandra McLellan, a professor at UWM's School of Freshwater Sciences said the team's ultimate goal is to be able to take sewage samples, generate and interpret the viral detection data, and pass the data along to public health. Following the apparent pattern in the WBE space at the moment, the methods development is essentially happening in parallel with application of the testing.

The team started early on in the pandemic, McLellan said, archiving samples and beginning the troubleshooting process for every possible variant of a typical workflow. Fortunately, although many labs in the field are using different methods, they all use similar controls, so through collaboration, "We can crowdsource the optimizing … so we can pivot more rapidly than if we were doing this in isolation," she said.

At the moment, the UWM lab is finding SARS-CoV-2 in the Milwaukee area at the limits of detection, while samples from hotspots in the US are yielding higher levels of virus.

McLellan noted that there are other labs in the US looking into the nitty gritty topics like viral molecular biology in sewage or PCR inhibition, as well as labs that are attempting to develop the best methods to correlate sewage SARS-CoV-2 to health indicators.

Meanwhile, her UWM lab has already begun surveilling nearly every county in Wisconsin through a collaboration with the Wisconsin State Lab of Hygiene and plans to continue this surveillance for one year, potentially providing one of the most comprehensive datasets on a statewide level.

McLellan said she also thinks other states and municipalities will soon jump into this type of monitoring. The lab's sample space overlaps La Crosse, too, and Gundersen's Kenny said the teams have been in touch, noting that the qPCR-based approach might be "much more scalable" than his current NGS efforts focused on a single city.

"I'm enthusiastic about NGS as it offers the potential to provide quantitative information on substrains and not just total disease burden in a community," Kenny said, "But, given limited resources, the more straightforward qPCR approach is more suitable for a broader regional approach."

McLellan said one reason there is so much basic research happening in parallel with attempts to enact projects to collect actionable data is that scaling WBE must take into account local factors. Labs need to collect data on coronavirus cases by zip code and overlay that with maps of the service area of the wastewater pipes, also called catchment areas, that feed the pipe where the sample was collected.

"It's all in the sampling," McLellan said. "Every city is different and every system is different, so we really need to partner with wastewater engineers, [and] the big leap between data and public health is that gap — understanding what city-specific or system-specific parameters need to be considered," she said.

Meanwhile, in Arizona, AquaVitas spun out of Halden's ASU lab in May of 2019 and has quickly pivoted to SARS-CoV-2 WBE.

"Our expertise is in the data analysis," said Co-founder Gushgari, but he noted that the firm also offers customers study design, as well as testing. AquaVitas' services can cost as little as $7,000 per year for a single site with infrequent monitoring, he said, but the cost increases depending on the spatial and temporal granularity desired.

The city of St. Augustine, Florida is one current customer for AquaVitas' SARS-CoV-2 surveillance, Gushgari said. "We provide them a report and we also do some consulting services … to dissect the data and help them understand what it actually means," he said. The nearby city of Palm Coast, Florida may also be getting on board, with city leaders suggesting this month that the projected $47,000 cost for 13 weeks of WBE monitoring at six sites would likely be covered through CARES Act funding.

Another new company, MIT spinout BioBot Analytics, claims to be the first company in the world to commercialize data from sewage. Although the Boston-based firm started out in 2018 focusing on opioid monitoring, it has also pivoted, publishing preliminary data in April showing it could detect SARS-CoV-2 in wastewater using RT-qPCR.

Earlier this month, BioBot demonstrated that SARS-CoV-2 detection in Boston-area sewage foreshadowed clinical case increases by as much as 10 days. Similar results were recently shown for the New Haven community in a report from researchers at Yale University, who found detection of the virus in sewage sludge was a seven-day leading indicator of positive testing and could provide three days of lead time on local increases in hospitalizations.

BioBot now purports to be working with approximately 400 facilities in 42 US states, covering more than 10 percent of the US population, according to a representative. One early BioBot customer, the city of Carmel, Indiana, reportedly expects the service will help indicate the health of residents down to the level of specific buildings.

Other recent WBE-related commercial efforts include a collaboration between lab services provider Source Molecular and LuminUltra, a firm that has developed a portable qPCR system. The partners are now offering environmental SARS-CoV-2 testing, including wastewater system monitoring. AquaVitas' Gushgari said he was also aware of local environmental testing firms that are marketing SARS-CoV-2 sewage testing to cities and towns.

But, overall, WBE data needs to be seen in the context of, and as a supplement to, other diagnostic and public health information. "It's not the end all. It's not a silver bullet. It is an important dataset, but it is not the only one that should be considered," Gushgari said.

Furthermore, there are also important legal and ethical questions pertaining to privacy and personal autonomy that societies may wish to hash out, even as the method is being broadly applied.

Still, coupling all the disparate WBE activities with the tremendous and ever-increasing global public health need, it is clear that increased funding support and a coordinated federal response could also be quite useful.

"There is not a collective effort nationally, but there should definitely be one," said UWM's McLellan. To date, "It is all grass roots," she said. "I literally started this [project] myself and went looking for grant funding."

McLellan said the onus has been on academics, in part because a wastewater treatment plant isn't likely to be able to start up this type of testing and public health departments don't have the expertise. Water science researchers are sort of a liaison or conduit, and the impetus must come from them.

Yet, "It would be great if there was a national effort, because funding always pushes the science forward," McLellan said. Academia is also unlikely to be the best site in the end for large-scale or long-term monitoring, and McLellan suggested that commercial testing labs may be the ultimate home for this kind of surveillance work.

At the National Academies' WTSB meeting, David Sedlak, an environmental engineer at University of California, Berkeley also emphasized that much more needs to be done.

"Researchers at universities and independent laboratories are doing monitoring of sewers and wastewater treatment plants in the US, Europe, and other countries," he said, "But this is in some ways a bit of a shoestring operation when you consider the magnitude of the challenge of the pandemic."

Emergency funding from NSF, Sedlak said, has allowed researchers to rapidly ramp up capacity, but the capacity is still limited. One important question now faces the WBE field in the US: "In a country that has some of the most advanced medical science and public health infrastructure in the world, what could we be doing beyond what is likely to happen through the efforts of a handful of determined university researchers?"

Federal involvement, funding for larger initiatives, and efforts of national and private labs could help ramp up the scale of WBE research, Sedlak said.

ASU's Halden also wonders why more areas aren't yet using this inexpensive tool that can provide so much information. Unfortunately, "The study of wastewater-based epidemiology resides in scientific no-man's land at the interface between human medicine, epidemiology, and environmental engineering," he said. "This has impeded both acceptance and funding of this important field of study."

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